

The University of Chicago

THE CONDITION OF CALCIUM, PHOS-
PHORUS, AND NITROGEN IN
EVAPORATED MILK

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS AND
HOUSEHOLD ADMINISTRATION

1936

By
UNA LANE ROBINSON

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THE CONDITION OF CALCIUM, PHOSPHORUS, AND NITROGEN IN EVAPORATED MILK

The effect of heat upon various constituents of fluid milk has been studied in some detail and it is generally acknowledged that the time and the temperature of heating are primary agents in producing the changes that occur during heating. However, the methods of study, the time periods and the temperatures used have been so varied that there are not sufficient data to show consecutive changes of all the constituents from the low temperature, short period heating to the high temperature, long period heating and many of the commonly accepted statements about these changes are based upon analogous reasoning rather than upon accumulated data. Since there have been fewer reports of work done at temperatures above boiling than at the lower pasteurization temperatures, little data are available concerning changes in the solubility and dispersion or partition of certain constituents of evaporated milk.

Because evaporated milk is now gaining such wide use where other milks have been used unsuccessfully, and because it is displacing bottled milk to an important extent in other situations, this work was undertaken in order to add some data concerning the nature of calcium, phosphorus and nitrogen in evaporated milk to that which is available for raw, boiled and pasteurized milks. That the changes in these constituents of evaporated milk would be great might well be assumed, for, though comparatively low, the long heating period for forewarming and evaporation, and the high temperature for sterilization which follows are the very conditions that would logically be expected to produce maximum changes or losses, if such changes were dependent only upon heating time and temperature.

REVIEW OF LITERATURE

Character of Raw Milk

Although this study is concerned with the condition of

calcium, phosphorus and nitrogen in evaporated milk, it seems pertinent to describe briefly the complexity of raw milk so that a background may be outlined against which differences found in evaporated milk may be measured.

The first evidence that milk is a very complex material is found when figures for its percentage composition are sought. It is then found that no two milks are exactly alike and minimum and maximum figures, as well as the average figure, are usually reported for each constituent. Reported analyses of milks give the following ranges for the proximate composition: protein 3.13 to 5.71 per cent; casein 2.65 to 4.5 per cent, usually averaging 76 to 80 per cent of the total protein; fat 3.55 to 4.94 per cent; and ash 0.68 to 0.96 per cent of the whole milk. These wide variations are induced by such factors as breed (Eckles and Shaw, 11; Overman, et al., 26), individuality (Eckles and Shaw, 11), morning or evening milk (Eckles and Shaw, 12), quarter of udder from which the milk is drawn (Proks, 28), phase of lactation (Eckles and Shaw, 10), quality and quantity of food (Cary and Meigs, 4; Eckles and Palmer, 13; Savage, 33), weather conditions (Rogers, 31), and exercise and excitement of the cow. However, although no two milks are alike, extreme differences are smoothed out when herd milks are compared.

Although herd milks are similar in percentage composition, other factors than percentage composition are effective in making milk a complex substance and any evaluation of its complexity must involve a consideration of its physical equilibria, for it is a poly-phased colloidal system in which each phase differs in its degree of dispersion. The continuous phase may be considered homogeneous for it is a water solution of lactose, certain sodium, potassium, magnesium, calcium, phosphorus, chlorine and citric acid salts and soluble proteins. The suspended phases are fat, calcium caseinate and some colloidal calcium phosphate. Each solid phase is suspended in the continuous phase in a very fine state of subdivision, the size of which has an important influence on the final equilibrium. Holm (Rogers, 31) has prepared a table from the results of many workers which shows something of the dispersion of the colloidal particles according to their size. If particles are less than 1 milli-micron in diameter they are truly soluble, as lactose and the soluble salts. Particles ranging in diameter from 1 to 10 milli-microns are below the ultramicroscopic

range but are not truly soluble. This range includes the particles of lactalbumin and lactoglobulin. Particles from 10 to 100 milli-microns in diameter are within the ultra-microscopic range, while those from 100 to 200 milli-microns are within the microscopic range. Since casein and colloidal calcium phosphate particles range from 1 to 200 milli-microns in diameter, they are found in the last three ranges. The fat globules are relatively very large, ranging in diameter from 1 to 10 microns in diameter and are completely in suspension.

The dispersion of the nitrogenous constituents is important for lactalbumin and lactoglobulin are usually considered as soluble proteins, which is not strictly true. It is true in the sense that they form transparent solutions, but although lactalbumin passes through clay filters after adsorption of the first portions, lactoglobulin is almost completely retained. In fact, Van Slyke and Bosworth (40) have shown that when milk is filtered through clay filters considerable quantities of the albumin which is present in the milk also fail to pass through the filter, being adsorbed on the retained casein. In other respects both of these proteins must be considered colloidal in nature.

The casein, too, is wholly colloidal and is suspended as calcium caseinate, which can be completely separated from the serum by using a semi-permeable membrane. Van Slyke and Bosworth (40) have shown that casein contains 0.71 per cent phosphorus and have calculated the calcium equivalents with which it combines and have found that there are four calcium atoms in each caseinate molecule. Now, although some calcium is combined with casein, there is much of the total calcium of the milk that is colloidal but not bound to the casein.

Whether the non-casein calcium is present as colloidal di- or tri-calcium phosphate, and whether some of the phosphate is bound to the colloidal protein have been problems of investigation for many workers. Soldner (35) was of the opinion that the calcium phosphate was present as tri-calcium phosphate. However, Van Slyke and Bosworth (40) believed it to be present as di-calcium phosphate and Palmer (27) is in accord with the latter view. Actually, no indisputable evidence has been presented to prove or disprove either statement. However, Whittier (45) has calculated the equilibrium concentrations of calcium and di-phosphate ions and believes that there are not enough such ions in milk to satu-

rate it with di-calcium phosphate.

The percentages of the minerals in milk are usually determined by an analysis of the milk ash which commonly consists of the carbonates, sulfates and phosphates of the metallic elements present in the milk. Now since much of the mineral of the ash, particularly the sulfur and the phosphorus, are from organic as well as from a inorganic sources, it is an unsound practice to use the data from ash analysis as a basis for setting up salt distributions. Even if these factors did not complicate the problem, some of the chlorine may be lost by incineration, and all of the citric acid is completely oxidized. Although the analysis of the ash cannot reveal the salt combinations within the milk, nevertheless, its own reaction is important and any change in it may affect the coagulation properties of the milk.

The salt combinations and their ionic concentrations together with the proteins, particularly casein, determine the buffer capacity of the milk. Changes in the buffer value may be brought about by differences in kind and quantity of either of these constituents as well as by differences in either the pH or titratable acidity of the milk; and will cause shifts in the equilibrium of many constituents which may produce undesirable effects when milk is heated. The pH value has been determined often and ranges from 6.3 to 7.4 for whole milk, the commonly accepted value average being 6.6. These values are markedly affected by the level of carbon dioxide in the milk, which may be lowered by exposure to the air, or by displacement when the hydrogen is bubbled through it when the hydrogen electrode is used. The titratable acidity of milk is also often reported, but it must be realized that the titratable acidity cannot be interpreted to measure more than the quantity of alkali necessary to shift the original pH to a pH which has been arbitrarily selected as the 'end point.' This is usually the pH at which phenolphthalein changes color. Rice (30) found that a decrease in titratable acidity was accompanied by a decrease in specific gravity, total solids, solids-not-fat, lactose, protein casein and phosphorus oxide. He points out also that milks of high acid content have greater buffer value than milks with low acid content.

Although the buffer action is responsible for many of the typical reactions encountered in the chemistry of milk, far too little is known of the buffer value of milk. It may be calculated

by a formula developed by Van Slyke (38) which is dependent upon the determination of the pH within short titration ranges and by plotting a titration curve the buffer index may be calculated for every pH. The equation used is

$$B = \frac{\Delta B}{\Delta pH} = \frac{(\text{cc acid or base used in titration}) (\text{Normality})}{(\text{Average volume over range involved}) (\text{pH change produced})}$$

where ΔB is the fraction of a gram equivalent of KOH added and ΔpH is the change in the pH value. In these terms a solution has a buffer value of 1 when a liter will take up one gram equivalent of strong acid or alkali per unit change in pH. When this formula is applied to calculate the buffer value of casein solutions whose titration curves are found in the literature, many discrepancies are found in the order of magnitude of the $\frac{\Delta B}{\Delta pH}$. Clark (Rogers, 31) points out that these discrepancies may be attributed to the false assumption that milk is a homogeneous system. Whittier (44), too, points out that so little is known of the buffer value of the individual buffer constituents of milk that much work is still necessary before any true interpretation of buffer value can be arrived at.

The Effect of Heat on Certain Milk Constituents

Much of our information about the effects resulting from the heating of milk has been acquired through the use of purified milk constituents rather than the milk itself. The necessity for resorting to this practice is readily understood when one considers again the complexity of milk. Thus, data have been acquired which must be used with considerable caution when interpreting the reactions of whole milks and not pure milk constituents. Many of the studies of the effect of heat on the milk proteins, particularly casein and albumin, have been carried out on the purified substances. The hydrolytic effect of heat on calcium caseinate is reported in an extensive literature. The calcium salt and the hydrolytic product, casein, show distinctly different characteristics. Sevdberg, Carpenter and Carpenter (34) have shown that the casein molecule doubles its molecular weight at 40°C., that is, heat causes a polymerization, which is not in line with Wright's (47) report that heating casein for thirty minutes to 120°C. had no effect on its constitution. Nor did Nichols (24) find any difference in the particle-size distribution when calcium

caseinate was heated to 65° and 95°C., thus indicating no polymerization of caseinate. Casein in milk will coagulate when heated, the rate of coagulation being dependent upon the time and temperature of heating. Of course, in milk, other factors than heat are effective and will be described later.

Other important studies of the milk proteins include work on the coagulation of lactalbumin. Table I summarizes some of the more important literature concerned with the coagulation of lactalbumin by heat. From this table it is clearly evident that within the ranges reported, the higher the temperature the more lactalbumin is coagulated. The time influence shows up from this table also, although most of the heating was done for the same period. But at the lowest temperature reported coagulation did take place although it required five hours of heating.

TABLE I
THE EFFECT OF HEAT ON THE COAGULATION OF LACTALBUMIN

Investigator	Heating Temperature	Heating Time	Observations
	°C.		
Orla-Jenson (25).	60	5 hrs.	Some coagulation
Babcock (1).....	65	20 min.	No change
Freudenreich (15)	68-69.5	30 min.	15 to 20 per cent coagulated
Rupp (32).....	62.8	30 min.	No coagulation
	65.6	30 min.	5.75 rendered insoluble
	68.3	30 min.	12.75 per cent coagulated
	71.1	30 min.	30.78 per cent coagulated
Weinlig (43).....	60	30 min.	8.5 becomes insoluble
	80	60 min.	40.0 per cent becomes insoluble

In 1888 Soldner (35) pointed out that calcium phosphate was lost from milk when it was heated. This has been confirmed by many investigators since that time, and much work has been done to determine how the salt equilibrium is altered by this precipitation. Soldner believed that di-calcium phosphate passed to tri-calcium phosphate and was precipitated in that form. This was the opinion of Milroy (22) also, and of Daum (5). Palmer (27), however, prepared a solution of colloidal di-calcium phosphate and found that large quantities of the salt were precipitated when the solution was heated and that it had not become tri-calcium phosphate. He, therefore, believes that the fixation of

calcium phosphate of milk is explained on the grounds of the effect of heat on the colloidal solution. It is certainly true, as seen from Tables II and III that very little of the filterable calcium or phosphorus is lost by heating to the pasteurization temperature.

TABLE II
REPORTED CHANGES IN THE TOTAL CALCIUM AND PHOSPHORUS CONTENT
OF MILK WHEN HEATED

Investigator	Temperature of Heating	Time of Heating	Observations
Soldner (35)	°C. 100		17.5-24 per cent calcium lost 10 -17 per cent phosphorus lost
Purvis, Brehaut and McHattie (29)	100	30 min.	Heated in a current of steam 3.5 per cent calcium lost 6.1 per cent phosphorus lost
	100	10 min.	Heated in open vessel 10.35 per cent calcium lost 12.6 per cent phosphorus lost
	100-113	30 min.	Autoclaved 5.3 per cent calcium lost
	100		Just boiled. 6.8 per cent calcium lost
Daum (5)	95	30 min.	11.4 per cent calcium lost 9.1 per cent phosphorus lost
	106-110	6 min.	6.1 per cent calcium lost 7.5 per cent phosphorus lost
Milroy (22)	Below boiling	60 min.	12.8 per cent calcium lost
DeVries and Boekhaut (6)	100		3.5 mg. per 100 cc. lost
Solomin (36)	80	30 min.	Slight precipitation of phosphorus

From the results reported in this table it is indeed difficult to formulate a precise statement about the losses encountered when milk is heated. The work of Purvis, Brehaut and McHattie seems to indicate that exposure to atmospheric conditions has more influence than has the temperature at which the milk is heated. The unquestionable fact is that both calcium and phosphorus are precipitated when milk is heated.

Other workers have approached the problem by trying to

TABLE III
REPORTED CHANGES IN THE SOLUBLE CALCIUM AND PHOSPHORUS
CONTENT OF MILK WHEN HEATED

Investigator	Temperature of Heating	Time of Heating	Observations
Rupp (32)	62.8	30 min.	No loss in either calcium or phosphorus
	68.3	30 min.	
Bell (2)	77.7	30 min.	Very small loss, exceeding experimental error only slightly
Grosser (16)	100	5 min.	5.2 per cent calcium lost
	100	15 min.	7 per cent increase in phosphorus
	100	30 min.	12.2 per cent calcium lost 1.1 per cent phosphorus lost 11.9 per cent calcium gained 3.5 per cent phosphorus gained
Magee (18)	65	30 min.	14 per cent calcium rendered inactive when precipitated with $\text{Fe}(\text{OH}_3)$
	70	30 min.	2 per cent calcium lost from rennin whey
	100	60 min.	4 per cent calcium lost from rennin whey
Diffloth (7)	60	30 min.	26 per cent phosphorus lost

find how much of the soluble elements are precipitated during the heating process. Table III reports the results of the most important investigations in this field.

Little work is reported on changes effected at boiling temperatures. Grosser reported the loss of serum calcium in 1913 and his work is often mentioned, yet it should be examined critically. Reference is usually made to the loss he observed after fifteen minutes of heating. He calculated this to be 5.4 per cent in the following way. He computed the calcium of the raw and the heated milk serums as percentages of the total calcium of the milk, and found them to be 23.4 and 18 per cent respectively. He then subtracted the one from the other and reported a loss of 5.4 per cent. Now if the calcium of the heated serum is calculated as per cent of the calcium of the raw serum it is found that 12.2 per cent of the serum calcium has been lost. No mention is made of the fact that when the milk was heated for thirty minutes the average difference was a gain rather than a loss. Two

of the milks that were heated did show losses, one of only 3.4 per cent, the other of 17 per cent; but the average difference was a gain of 11.9 per cent for calcium. Nevertheless, even with these irregular results it has been generally concluded from this work that serum calcium is lost from milk when it is heated. The fact that at the end of five minutes heating there was an apparent gain of 7 per cent of phosphorus, which dropped to 3.5 per cent at the end of thirty minutes is also generally ignored.

Of those reports of studies of changes at boiling temperatures, the work of Harvey and Magee should be mentioned. They did not work with serum, but with the filtrate obtained after treatment with various precipitants. Thus they found that 14 per cent of the calcium failed to react with colloidal ferric hydroxide after heating; 2 per cent and 4 per cent respectively were lost from the rennin whey at 30 and 60 minutes heating at 70 and 100°. The work of Rupp on the effect of pasteurization on the calcium and phosphorus of milk serum seems to be about the only work that withstands criticism. At those temperatures and heating periods there was no measurable loss. In a brief report which carries no figures Dutcher (9) also says, "No evidence was obtained to support the contention that calcium and phosphorus are changed to insoluble forms during the heating process."

Losses other than mineral losses, particularly carbon dioxide, are encountered when milk is heated. Loss of carbon dioxide causes the pH to rise. However, as the temperature continues to increase and as the heating period is prolonged, the pH drops again because of the formation of acid from the milk constituents. Whittier and Benton (46) have reported on this effect. They found also that the rate of increase in the formation of acid diminished during coagulation and attributed it to readjustment of the buffer constituents, the detail of which has not yet been explained.

Although coagulation is the one visible change that occurs when milk is heated, very little is understood about the coagulation mechanism. The rate of coagulation is dependent upon concentration, temperature and time of heating. Moreover, the time of coagulation at any one given temperature depends also upon the temperature and the time of previous heating. If milks are concentrated until they contain about 20 per cent milk solids-not-fat the stability is markedly increased if the milk has been

previously heated. The time and temperature of forewarming are also important. Stability is increased up to 100° . Improvement also increases with time up to thirty minutes at 95°C . If higher temperatures are used the same improvement can be attained in a shorter period. Homogenization also affects the stability. Maximum stability is produced when the milk has been preheated and homogenized at 80° and minimum stability is encountered if the preheating and homogenization takes place at 60° .

Probably more important than either of these factors is the ionic equilibrium of the salts and the way it affects the calcium caseinate. Somner and Hart (37) studied this problem and decided that casein required a "definite optimum calcium content for its maximum stability" and that there was a critical balance between the calcium content of casein on the one hand, and magnesium, citrates and phosphates on the other. They also found that, although the acid fermentation affects the stability of milk, it could not be used as a criterion of stability for fresh milk varies so in its titratable acidity that the acid fermentation could not be measured. When certain buffer salts as phosphates, citrates or carbonates are added to milk they have a stabilizing effect which has been studied by Benton and Albery (3). They found that there is an optimum balance of pH and buffer salts, particularly citrates, and that within a pH range of 6.58 to 6.65 the salt balance is the more important factor, but that outside that range the pH has the greater influence on stability. Holm, Webb and Deysher (17) found that milks may be divided into three groups in respect to their stability to salts with strong negative ions. Type I becomes more stable when phosphates are added and less stable when chlorides are added. Type II is at optimum stability in its untreated condition and addition of either phosphate or chloride will reduce its stability. The stability of type III decreases by the addition of phosphate and reaches its maximum with an appropriate addition of chloride. One type merges gradually into the other, type I being the more common. Type III changes gradually to type I with storage, and the rate of change is accelerated with the development of lactic acid, although it may occur without measurable change in the pH, which indicates a shift in the equilibrium of the calcium phosphate and calcium caseinate. No technique has been developed whereby one can determine the type of the milk before sterilization.

All of the conditions just described have an important bearing on the processing of evaporated milk. Before milk is accepted for processing it must pass the odor test, which is done by an expert who judges its fitness by the odor that is developed within the shipping container. Undesirable milks develop characteristic unidentified volatile products. Milks from all sources are mixed and filtered and then passed into the forewarming chamber where they are held at the required temperature and time which has been previously determined to give the best product for the plant. These and the sterilization time and temperature, are the trade secrets of the individual condensaries. This forewarming stabilizes the milk for its later sterilization. At one time stabilizers were added to the milk before forewarming, thus putting into application the findings of Benton and Albery. This is no longer done. The next step in the treatment of the milk is that of evaporation. By this concentration of the milk solids the stability is again threatened, but is again readjusted by the homogenization. Homogenization is usually thought of as being practiced only for the purpose of breaking up the fat globules, but the work of Webb and Holm (42) and of Doan and Minster (8) shows that homogenization also stabilizes the milk to heat. After being homogenized, the concentrated milk is canned, sealed and sterilized. During the process of sterilization, the product becomes viscous even to the stage of forming a delicately tender jel. This condition is desirable from the producer's viewpoint, but the consumer will invariably reject a product that shows any coagulation. Therefore the cans are placed in a shaker and are vigorously agitated to break up any coagulum which may have been formed, thus restoring the product to a fluid condition.

Numerous nutrition experiments have shown that evaporated milk is more efficiently utilized by the human body than raw or pasteurized milk. Now since the same constituents are presumably present in both products, there must be some change either in the nature or distribution of some or all of the constituents. This brief survey of published data discloses that little indisputable evidence has been presented about changes effected by heating milk and that most of the data which is available applies to changes which take place at pasteurization temperatures and are not complicated by the process of homogenization.

PURPOSE

The purpose of this work, then, has been to study the changes in the partition of calcium, phosphorus and nitrogen of commercially evaporated milk by analyzing several series of fractions of the evaporated milk and of the raw milk from which it originated and comparing the two. The fractions have been separated primarily by mechanical means in order to avoid possible shifts which might be brought about by chemical separation. Only one fractionation of whole milk and a sub-fraction of one of the fractions have been done by chemical means.

EXPERIMENTAL WORK

The five pairs of milks, raw and evaporated, which are compared in this study were received from commercial condensaries. Two samples of evaporated milk were purchased in the open market and are compared with the averages of the raw samples used in the study.

The Raw Milk

The co-operating condensaries received the raw milk from the usual sources and mixed it in the usual manner. One gallon samples were then withdrawn from the large batch, placed in tins, sealed, packed in ice and sent immediately to the laboratory. Work on the first two samples was delayed until the morning following collection as they were received at the laboratory late in the evening. The other three milks were received by noon and work begun immediately. In all cases the milks remained in the ice until the work of sampling was begun. Afterward they were protected as much as possible by storing in a refrigerator. In only one case was souring encountered before fractionation and sampling was completed. That was the first milk, which soured before the serum could be sampled.

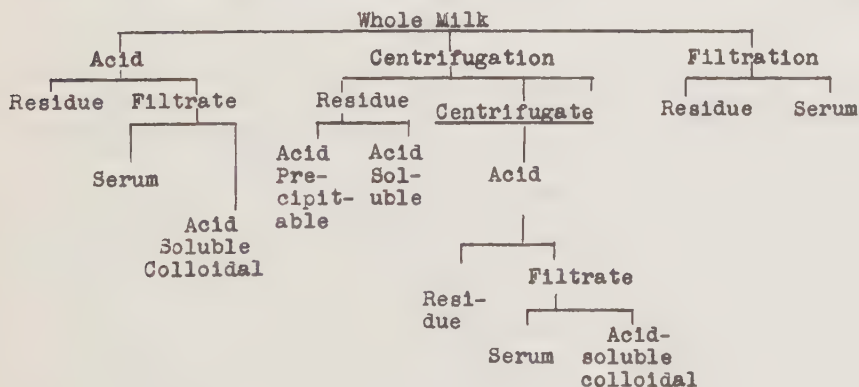
The Evaporated Milk

After the raw samples were removed from the large batch

of milk, it was subjected to the usual processes of commercial manufacture of evaporated milk. When the process was completed, several cans of the milk were sent to the laboratory by post. These samples were kept under room conditions until it was convenient to make the analyses. One part of the evaporated was then diluted with one part of distilled water, according to the recommendations commonly given by the condensaries and according to common family practice, except that families use tap water instead of distilled water. This reconstructed milk is referred to as 'the whole evaporated milk.'

The Fractionation of the Milk

Three portions of each whole milk, both raw and evaporated, were separated by different methods into two fractions, a liquid and solid fraction. From one portion the acid-precipitable constituents were precipitated by acetic acid and separated by filtering through filter paper, thus giving the two fractions, precipitate and acid filtrate. Another portion was separated into its two fractions by centrifugation, giving the fluid centrifugate and the solid centrifugal residue. The third portion was filtered through a Pasteur-Chamberland filter, giving the fluid serum and the residue, which may be distinguished from the other residues by designating it as the Pasteur-Chamberland residue. The centrifugate was further separated into sub-fractions by precipitating the acid-precipitable constituents as was done with the whole milk. Now since the truly soluble constituents are in the serum, if these constituents are subtracted from the constituents of the



other fluid fractions and sub-fractions, other interesting sub-fractions can be observed. The preceding genealogical tree will serve to show more concisely the relationships of these fractions to each other.

These fractions of the two milks are compared and their differences discussed. Figures for a number of the fractions are obtained by difference rather than by actual chemical analyses. Nitrogen, phosphorus and calcium analyses were made of the whole milks, the centrifugates and the serum. Residues were determined by difference. Nitrogen analyses were made on the acid-precipitable residues of the whole milk and the centrifugate. Acid soluble nitrogen, or nitrogen that was not precipitated by acid, was determined by difference. Phosphorus analyses were made on the acid-filtrate of the whole milk and the centrifugate. Phosphorus in the acid residues was determined by difference. Since each acid-filtrate contained the truly soluble constituents as well as those constituents which were colloidal but made soluble by acid, the latter was determined by difference. The residue obtained from centrifugation contains only colloidal material, but this is of two types: that which is precipitable by acid and that which is not precipitable. The acid-precipitable part of the residue is obtained by subtracting the acid-precipitable residue of the centrifugate from the total acid-precipitable constituents. The acid-soluble portion of the centrifuged deposit, then, is the residue remaining after this subtraction. Another way of determining the acid-soluble fraction of the centrifuged deposit is to subtract the acid soluble part of the centrifugate from the total acid-soluble fraction.

Techniques of Mechanical Separation

Centrifugation.--The apparatus used for centrifugal separation was a Sharples supercentrifuge. In structural principle the apparatus resembles a miniature cream separator. It subjects the material to a centrifugal force that is 40,000 times that of gravity. Thus only truly soluble or very minute colloidal micelle remain in the liquid centrifugate.

Two techniques were used in the centrifugation process. The market milks were measured and introduced into the bowl until the milk began to flow from the cover. The cover discharge was then collected in the same container which was supplying the bowl

with milk. Thus the same milk was continually returned to and discharged from the apparatus. At the end of the period, the liquid remaining in the bowl was collected with that which was received from the cover. These two milks did not have as much of the colloidal material removed as it was felt it might be possible to remove. So the technique was modified for the paired milks which were centrifuged later.

The second technique of centrifuging involved supplying milk to the bowl until it began to flow from the cover, then shutting off the supply and centrifuging only that portion which was caught in the bowl. When the liquid was removed from the bowl after one hour it had lost much more of the colloidal material than had the milk centrifuged by the other technique.

The residue was removed from the inside of the tubular bowl by scraping into a tared dish. Although the residues were analyzed the results are not reported for the material could not be quantitatively recovered.

In order to determine how long a sample of milk should be centrifuged by the second technique to remove the most colloidal material that was practicable, samples of the same milk were centrifuged for different periods, and then analyzed. Table IV shows that the loss in the second thirty minutes of a sixty minute period was slight and that therefore a sixty minute period was sufficiently long and might even be diminished if necessary.

TABLE IV
EFFECT OF LONG AND SHORT CENTRIFUGAL PERIODS IN REMOVAL OF
THE COLLOIDAL CONSTITUENTS OF MILK

	Period of Centrifuging					Per cent Lost in Second 30 Minutes
	None	Thirty Minutes		Sixty Minutes		
	Per cent Whole Milk	Per cent Centri- fugate	Per cent Loss	Per cent Centri- fugate	Per cent Loss	
Nitrogen	0.5645	0.3270	42.07	0.3230	42.78	0.71
Phosphorus	0.1173	0.0634	45.95	0.0578	50.72	4.77
Calcium	0.1237	0.0463	62.57	0.0405	67.26	4.69

The quantity of colloidal deposit varies also with the rate of speed of the centrifuge. The speed of this centrifuge

could not be controlled and was not checked. No doubt some of the variation of total deposit was due to this factor, for the electrical current was not synchronized and there was considerable fluctuation in the current, which would affect the speed of the centrifuge.

The bowl of the centrifuge always received 300 cubic centimeters of milk before any was discharged from the cover. The quantity recovered was approximately 250 cubic centimeters after centrifuging for one hour.

Filtration. Samples of each whole milk were filtered through porcelain filters, using a Pasteur-Chamberland apparatus. This apparatus requires considerable pressure to force the milk serum through the filter. The screw top of the cylinder was therefore fitted with a valve which in turn could be attached to a gauge so that the pressure, obtained from a hand pump, could be measured. When the pressure had been brought to sixty pounds, the valve was made air-tight, and the whole apparatus detached from the gauge and placed in a refrigerator for the filtration period. Several days were required to collect enough of the serum for analysis. The air would leak through the filter and the pressure had to be renewed several times during the filtration period.

Rupp (26) showed that the first portion of the serum had to be discarded because the filter adsorbed the first portion of the soluble materials as they passed through, equilibrium being reached when 50 to 70 cubic centimeters of the serum had passed through the filter. In order to determine how much of the serum should be discarded before equilibrium was established in the apparatus used for this study, some analyses of the successive fractions of the serum were run. Table V shows that it was neces-

TABLE V
CALCIUM CONTENT OF SUCCESSIVE FRACTIONS OF SERUM

Collection Period	Volume	Calcium Per 10 cc. Serum
Hours	cc.	mg.
24	50	2.55
48	35	3.35
72	10	3.42
96	20	3.92
120	10	3.89
144	10	3.90

sary to discard almost 100 cubic centimeters before the mineral content of the serum was constant. This loss reduced the usable volume so greatly that desirable pH determinations and titratable acidity data could not be obtained.

Because it required so many hours for filtration when the whole milk was placed in the filtration apparatus, some modification was sought that would reduce the filtration period. Only after the first four milks had been filtered in this way was a modification found that seemed acceptable. This consisted simply of centrifuging the milk before filtering it. Centrifuging cannot remove soluble material, therefore only that material which would clog the pores of the filter was removed and the filtration was hastened.

The candle was cleaned by scraping away the adhering material, drying in the drying oven and incinerating in a muffle furnace. After removing the organic material in this way, the ash was washed out by filtering distilled water through the candle in the usual way.

When the first samples were filtered, whole milk was used rather than skim-milk on the principle that the fat could not be removed from the evaporated milk and the two fractions would be more comparable. As a matter of fact, since it required so long for filtration, the fat separated in the raw milks and the effect was the same as though skim-milk had actually been used. Of course when the later samples were filtered after being centrifuged they contained even less fat than a skim-milk would have contained.

Analytical Methods

Sampling.--All the fluid samples were pipetted from a 10 cubic centimeter pipette and the pipette was drained for one minute, a stop watch being used to check the time. After the samples were thus measured into their respective receptacles, three to five other samples were measured in the same way and weighed, the average being used as the weight of the samples when the percentages were calculated later. That this is justified is shown by Table VI. The greatest deviation from the average is 0.0027 grams, an error of 0.025 per cent.

TABLE VI
WEIGHTS OF TEN CUBIC CENTIMETER VOLUMES OF THE SAME
WHOLE MILK

Sample	Weight	Deviation from Average	Error
	gm.	gm.	per cent
1	10.7086	-0.0007	-0.006
2	10.7104	+0.0011	+0.010
3	10.7072	-0.0021	-0.019
4	10.7130	+0.0027	+0.025
5	10.7073	-0.0020	-0.018
Average	10.7093	-0.0002	-0.0016

Specific gravities.--A hydrometer was used in determining the specific gravity of the paired milks. The specific gravities of the two market milks were calculated from the weight of 10 cubic centimeters of the milk.

Hydrogen ion concentration and titratable acidity.--The hydrogen ion concentration of the two market milks, the first pair of milks and the second raw milk was measured with a hydrogen electrode, a Leeds-Northrup student potentiometer being used. This apparatus was also used for the few titration curves that were obtained. The hydrogen ion and titration values for the last milks were determined with the quinhydrone electrode.

Nitrogen.--The Arnold-Gunning (19) modification of the macro-Kjeldahl method was used for nitrogen determinations. The greatest percentage error was 2 per cent and this large error was only encountered in those fractions that were very low in nitrogen. The nitrogen of casein and other precipitable fractions was determined by the same method after separation with acetic acid.

Phosphorus.--The phosphorus of the first four milks and of the raw samples of the fifth milk was determined by the method of Neumann (23) and the precautions advocated by McCandless and Burton (20) were practiced. The largest error encountered in these determinations was 2 per cent and when found was always in samples of low phosphorus content. The Fisk and Subbarow (14) method for phosphorus determinations was used for the last milks. Determinations were done in triplicate.

Calcium.--The potassium permanganated titration modifi-

cation of McCrudden's (21) method for calcium was used. Determinations were done in triplicate and the greatest error was not larger than 2 per cent.

RESULTS AND DISCUSSION

The analytical data are reported in Tables VII, VIII, and IX. The two market milks 1 and 2, had no raw pairs with which they can be compared. Therefore the average for the five raw milks is used when it is necessary to compare these evaporated milks with raw milks. This practice affects a number of calculations, and it seems logical, therefore, to calculate two averages for the evaporated milks, (a) the average for all milks, which is influenced by the practice just described, and (b) the average for the paired evaporated milks. These averages are closely alike in all cases and any final interpretation involving averages can be based upon either average without changing significantly the final conclusions.

Analysis of these tables reveals that the percentage of each constituent in each whole evaporated milk is greater than in its paired raw milk. This result is brought about, of course, because the volume of water used for the dilution of the evaporated milk is not as large as the volume of water that has been removed from it by evaporation. This seeming increase in all the constituents masks some of the changes that take place because of heating and homogenization only. It is important, nevertheless, that these mass changes resulting from so many factors be considered for, after all, the evaporated milk is used at the dilution at which the analyses were made. However, if these data are compared with the data of other workers who have considered only the effect of time and temperature of heating, they must rest upon a basis common to that used by those workers. This common factor is an equal water content of both raw and heated milks. It is desirable, therefore, to know what the percentage of the constituents, which are being considered in this study, would have been if the evaporated milk had been diluted with a volume of water that was equal to the volume which had been evaporated from it. Since the water content of neither milk was determined, another constituent whose percentage in both milks was determined and whose weight will not vary when subjected to the conditions of manu-

TABLE VII
THE DISTRIBUTION OF NITROGEN IN RAW AND EVAPORATED MILKS

Milk Number	Raw Milk		Evaporated Milk					
	Mg. per 100 cc.	Per cent of Total Raw	Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
			Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Total Nitrogen in Whole Milk								
1			571	110.87	100.00	515	100.00	100.00
2			559	108.54	100.00	515	100.00	100.00
3	489	100.00	571	116.77	100.00	489	100.00	100.00
4	489	100.00	576	117.79	100.00	489	100.00	100.00
5	535	100.00	585	109.35	100.00	535	100.00	100.00
6	521	100.00	586	112.48	100.00	521	100.00	100.00
7	542	100.00	560	103.32	100.00	542	100.00	100.00
AAM ¹			573	111.30		515	100.00	100.00
APM ²	515		576	111.94		515	100.00	100.00
Acid-Precipitable Nitrogen in Whole Milk								
1			469	91.07	82.14	423	82.14	82.14
2			453	87.96	81.04	417	80.97	80.97
3	341	69.73	479	97.96	83.89	410	83.84	83.84
4	336	68.71	478	97.75	82.99	406	83.03	83.03
5	396	74.02	487	91.03	83.25	445	83.18	83.18
6	415	79.65	534	102.49	91.13	475	91.17	91.17
7	413	76.20	514	94.83	91.79	497	91.70	91.70
AAM			487	94.73	85.18	439	85.15	85.15
APM	380	73.61	498	96.81	86.61	446	86.58	86.58
Non-Precipitable Nitrogen in Whole Milk								
1			102	19.81	17.86	92	17.86	17.86
2			106	20.58	18.96	98	19.03	19.03
3	148	30.27	92	16.77	16.10	79	16.16	16.16
4	153	31.29	98	20.04	17.03	83	16.97	16.97
5	139	25.98	98	18.32	16.62	90	16.63	16.63
6	106	20.35	52	9.98	8.87	46	8.83	8.83
7	129	23.80	48	8.86	8.57	45	8.30	8.30
AAM			85	16.34	14.86	75	14.83	14.83
APM	136	26.34	78	15.79	13.44	69	13.38	13.38
Serum Nitrogen								
1			31	6.02	5.43	28	4.90	4.90
2			46	8.93	8.23	42	7.51	7.51
3			28	5.43	4.90	24	4.91	4.91
4	28	5.73	38	7.77	6.60	32	6.54	6.54
5	47	8.79	46	8.60	7.86	43	8.04	8.04
6	43	8.25	32	6.14	5.46	29	5.57	5.57
7	36	6.64	43	7.93	7.68	42	7.75	7.75
AAM			38	7.26	6.69	34	6.47	6.47
APM	39	7.85	40	7.61	6.90	37	6.98	6.98

¹AAM, average for all milks.

²APM, average for paired milks.

³Figures for unpaired milks in these columns based on average of paired raw milks.

TABLE VII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc.3	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Colloidal Nitrogen in Whole Milk								
1			540	104.85	94.58	487	94.56	94.56
2			513	99.61	91.77	473	91.84	91.84
3			543	105.44	95.10	465	95.09	95.09
4	461	94.27	538	110.02	93.40	457	93.46	93.46
5	488	91.21	539	100.75	93.31	492	91.96	91.96
6	478	91.75	554	106.33	94.54	492	83.96	83.96
7	506	93.36	517	95.39	92.32	500	92.25	92.25
AAM ¹			535	103.20	93.57	481	93.39	93.39
APM ²	483	92.65	537	103.12	93.39	485	90.41	90.41
Acid Soluble Colloidal Nitrogen of Whole Milk								
1			71	13.79	12.43	64	12.43	12.43
2			60	11.65	11.07	46	8.93	8.93
3			64	12.43	11.21	55	11.25	11.25
4	125	25.56	60	12.27	10.42	51	10.43	10.43
5	92	17.20	52	9.72	8.87	46	8.79	8.79
6	63	12.09	20	3.84	3.41	17	3.26	3.26
7	93	17.16	3	0.55	0.54	3	0.55	0.55
AAM			47	9.17	8.28	40	7.95	7.95
APM	93	18.00	34	6.59	5.81	29	5.61	5.61
Nitrogen in Centrifugate								
1			356	69.13	63.68	328	63.46	63.46
2			275	56.23	48.16	236	48.26	48.26
3	206	42.13	315	64.41	54.69	267	54.60	54.60
4	185	37.83	313	58.50	53.50	286	53.46	53.46
5	163	30.47	310	59.50	52.90	276	52.97	52.97
6	147	28.21	279	51.48	49.82	270	49.81	49.81
7	159	29.36	308	57.21	53.79	277	53.76	53.76
AAM			298	58.02	51.81	267	51.82	51.82
APM	172	33.60						
Acid-Precipitable Nitrogen in Centrifugate								
1			263	51.06	47.05	242	43.29	43.29
2			204	41.72	35.73	175	35.79	35.79
3	62	12.68	203	41.51	35.24	172	35.17	35.17
4	59	12.07	225	42.06	38.39	206	38.50	38.50
5	47	9.05	234	44.91	39.93	208	39.92	39.92
6	41	7.87	208	38.38	37.14	201	37.08	37.08
7	46	8.49	223	43.27	38.91	201	38.29	38.29
AAM			215	41.92	37.29	192	37.29	37.29
APM	51	10.03						

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in these columns based upon average for paired raw milks.

TABLE VII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Non-Precipitable Nitrogen of Centrifugate								
1			93	18.06	16.64	86	16.70	16.70
2			71	14.52	12.43	61	12.47	12.47
3	144	29.45	112	22.90	19.44	95	19.43	19.43
4	126	25.77	88	16.45	15.04	80	14.95	14.95
5	116	21.68	76	14.59	12.97	68	13.05	13.05
6	106	20.35	71	13.10	12.68	69	12.75	12.75
7	113	20.85	85	16.60	14.87	77	14.88	14.88
AAM ¹								
APM ²	121	23.62	84	16.31	14.51	75	14.53	14.53
Colloidal Nitrogen of Centrifugate								
1			310	60.19	55.46	286	55.55	55.55
2			247	47.96	43.26	212	43.35	43.35
3			277	56.65	48.09	235	48.06	48.06
4	157	32.11	267	49.91	45.64	243	45.42	45.42
5	116	21.68	278	53.36	47.44	247	47.41	47.41
6	104	19.96	236	43.54	42.14	228	42.07	42.07
7	123	22.69	269	51.93	47.00	242	46.98	46.98
AAM								
APM	125	24.11	264	50.86	45.83	238	45.74	45.74
Acid Soluble Colloidal Nitrogen in Centrifugate								
1			47	9.13	8.41	44	8.57	8.57
2			43	8.79	7.53	37	7.57	7.57
3			74	15.13	13.26	63	12.88	12.88
4	98	20.04	42	7.85	11.79	37	6.91	6.91
5	69	13.29	44	8.44	7.51	39	7.49	7.49
6	63	12.09	28	5.17	5.00	27	4.98	4.98
7	77	14.64	47	9.09	8.92	41	7.97	7.97
AAM								
APM	77	15.01	47	9.15	9.39	42	8.07	8.07
Total Nitrogen Removed by Centrifuging								
1			203	39.42	36.31	187	36.31	36.31
2			296	60.53	51.84	253	51.74	51.74
3	283	57.87	261	53.37	45.31	222	45.40	45.40
4	304	62.17	272	50.84	63.59	249	46.54	46.54
5	372	69.53	276	52.98	47.10	245	47.02	47.02
6	374	71.85	281	51.85	50.18	272	50.18	50.18
7	383	70.66	265	51.66	46.20	238	46.20	46.20
AAM								
APM	343	66.35	277	53.91	51.60	248	48.18	48.18

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in these columns based upon average for paired raw milks.

TABLE VII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Acid-Precipitable Nitrogen Removed by Centrifuging								
1								
2			190	36.89	33.99	175	33.98	33.98
3	279	57.05	275	56.24	48.16	235	48.06	48.06
4	277	56.65	261	53.37	45.31	222	45.40	45.40
5	349	65.23	262	48.97	44.79	239	44.67	44.67
6	374	71.79	276	52.98	47.10	245	47.02	47.02
7	367	67.71	281	51.85	50.18	272	50.18	50.18
AAM ¹			257	50.06	44.75	231	44.88	44.88
APM ²	329	63.69	271	52.68	47.11	243	47.06	47.06
Non-Precipitable Nitrogen Removed by Centrifuging								
1								
2			13	2.52	2.33	12	2.33	2.33
3	4	0.82	21	4.29	3.68	18	3.68	3.68
4	27	5.52	00	0.00	0.00	00	0.00	0.00
5	23	4.30	10	1.87	1.71	9	1.68	1.68
6	00	0.00	00	0.00	0.00	00	0.00	0.00
7	16	2.95	00	0.00	0.00	00	0.00	0.00
AAM			7	1.45	1.29	6	1.28	1.28
APM	14	3.40	6	1.23	1.08	5	1.07	1.07

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in this column are based on average for paired raw milks.

TABLE VIII
THE DISTRIBUTION OF PHOSPHORUS IN RAW AND EVAPORATED MILKS

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw	Per cent of Total Evapo-rated	Mg. per 100 cc.	Per cent of Total Raw	Per cent of Total Evapo-rated
Total Phosphorus in Whole Milk								
1			102	106.25	100.00	92	95.88	100.00
2			104	108.33	100.00	96	100.00	100.00
3	92	100.00	107	116.30	100.00	92	100.00	100.00
4	92	100.00	109	118.48	100.00	93	101.08	100.00
5	93	100.00	103	110.73	100.00	94	101.07	100.00
6	107	100.00	126	117.76	100.00	112	104.67	100.00
7	95	100.00	119	125.26	100.00	115	121.05	100.00
AAM ¹			110	114.73	100.00	99	103.53	100.00
APM ²	96	100.00	113	117.70	100.00	101	105.57	100.00
Acid-Precipitable Phosphorus in Whole Milk								
1			21	21.87	20.59	20	20.83	20.83
2								
3	27	29.35	22	23.91	21.16	19	20.65	20.65
4	29	31.52	19	20.65	17.43	17	18.47	18.27
5	20	21.51	11	11.83	10.68	10	10.75	10.64
6	29	27.10	38	35.51	30.16	32	29.90	28.57
7	12	12.63	27	28.42	22.69	25	26.31	21.74
AAM			23	23.69	21.47	20	21.15	20.11
APM	23	24.42	23	23.70	20.45	20	21.22	19.97
Non-Precipitable Phosphorus in Whole Milk								
1								
2			83	86.46	79.81	76	79.17	79.17
3	65	70.65	85	92.39	79.44	73	79.35	79.35
4	63	68.48	90	97.83	82.57	76	82.61	81.72
5	73	78.49	92	98.92	89.32	83	89.25	88.30
6	78	72.90	88	84.11	70.31	80	74.77	71.43
7	83	87.37	93	97.89	78.93	90	94.73	78.26
AAM			88	92.93	80.06	79	80.30	79.72
APM	72	75.78	89	94.23	80.07	80	80.14	79.81
Serum Phosphorus								
1			38	39.58	37.25	34	35.42	36.95
2			40	41.67	38.46	37	38.54	38.54
3			32	34.78	29.91	27	29.35	29.35
4	45	48.91	38	41.30	34.86	32	34.78	34.41
5	48	52.69	34	36.56	33.01	31	33.33	32.98
6	54	50.46	40	37.38	31.25	35	32.71	31.25
7	63	66.31	62	65.26	52.10	60	63.16	52.17
AAM			41	42.37	36.69	37	37.89	36.52
APM	53	54.59	43	45.13	37.80	39	40.99	37.56

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in these columns based upon average for paired raw milks.

TABLE VIII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw	Per cent of Total Evapo-rated	Mg. per 100 cc. ³	Per cent of Total Raw	Per cent of Total Evapo-rated
Colloidal Phosphorus of Whole Milk								
1			64	66.67	69.59	58	60.42	63.04
2			64	66.67	66.67	59	61.46	61.46
3			75	72.64	81.52	61	70.65	70.65
4	47	51.09	71	77.17	65.14	61	66.30	65.59
5	44	47.31	69	74.19	66.99	62	66.67	69.96
6	53	49.53	86	82.24	68.25	77	71.96	68.75
7	32	33.68	57	60.00	47.90	55	57.89	47.83
AAM ¹			69	72.64	64.94	63	65.05	63.89
APM ²	44	45.40	71	73.40	62.07	64	65.71	63.05
Acid Soluble Colloidal Phosphorus in Whole Milk								
1			43	44.79	41.35	39	40.63	40.63
2			53	57.61	49.53	46	50.00	50.00
3			52	56.62	46.71	44	47.83	47.31
4	18	19.56	58	62.37	56.31	52	55.91	55.32
5	25	25.81	48	46.73	39.06	45	42.06	40.18
6	24	22.43	31	32.63	26.05	30	31.58	26.09
7	20	21.05	47	50.12	43.98	44	44.67	43.25
AAM			47	49.59	42.38	43	44.35	42.23
APM	22	22.21	47	49.59	42.38	43	44.35	42.23
Phosphorus in Centrifugate								
1			57	59.38	55.88	51	53.13	55.43
2			65	67.71	62.50	60	62.50	62.50
3	76	82.61	55	59.78	51.40	47	51.09	51.09
4	50	54.35	52	56.52	47.71	44	47.83	47.31
5	47	50.54	58	62.36	56.31	53	56.99	56.38
6	55	51.40	57	53.24	44.53	51	47.67	45.54
7	57	60.00	69	72.63	57.98	67	70.53	58.26
AAM			59	61.66	53.76	53	55.68	53.79
APM	57	59.68	58	60.91	51.58	53	54.82	51.72
Acid-Precipitable Phosphorus in Centrifugate								
1			14	14.58	13.46	13	13.54	13.54
2			9	9.78	8.41	8	8.69	8.69
3	17	18.48	7	7.61	6.42	6	6.52	6.45
4	1	1.09	12	12.90	11.65	11	11.83	11.70
5	5	5.38	12	12.90	11.65	11	11.83	11.70
6	5	4.67	13	12.15	10.32	12	11.21	10.71
7	3	3.16	15	15.79	12.61	15	15.79	13.04
AAM			12	12.13	10.48	11	11.26	10.82
APM	6	6.55	11	11.65	9.88	11	10.81	10.12

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in these columns based upon average for paired raw milk.

TABLE VIII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Non-Precipitable Phosphorus of Centrifugate								
1								
2			51	53.12	49.04	47	48.96	48.96
3	59	64.13	46	50.00	42.99	39	42.39	42.39
4	49	53.26	45	48.91	41.28	38	41.30	40.86
5	42	45.16	46	49.46	44.66	41	44.09	43.72
6	50	46.73	44	41.12	34.37	39	36.45	34.82
7	54	56.84	54	56.84	45.38	52	54.74	45.22
AAM ¹			48	49.76	42.95	43	44.65	43.65
APM ²	51	53.22	47	49.27	41.74	42	43.79	41.38
Colloidal Phosphorus of Centrifugate								
1			19	19.79	18.63	17	18.48	17.71
2			25	26.04	24.04	23	23.96	23.96
3			23	25.04	21.49	20	21.74	21.74
4	5	5.43	14	15.22	12.84	12	13.04	12.90
5	0	0.00	24	25.81	23.30	22	23.65	23.40
6	1	0.93	17	15.89	13.49	16	14.95	14.29
7	0	0.00	7	7.37	5.88	7	7.37	6.09
AAM			19	19.30	17.09	17	17.49	17.27
APM	2	0.47	15	17.61	15.40	15	16.15	15.68
Acid Soluble Colloidal Phosphorus of Centrifugate								
1			11	11.46	10.57	10	10.42	9.61
2			12	13.64	11.21	12	14.13	14.13
3	0	0.00	7	7.61	6.42	6	6.52	6.45
4	0	0.00	12	12.90	11.65	10	10.75	10.64
5	0	0.00	4	3.43	3.17	4	3.77	3.57
6	0	0.00	0	0.00	0.00	0	0.00	0.00
7	0	0.00	8	8.17	7.17	7	7.59	7.40
AAM			8	8.17	7.17	7	7.59	7.40
APM	0	0.00	7	7.52	6.49	6	7.03	6.79
Total Phosphorus Removed by Centrifuging								
1			45	48.87	44.11	41	42.71	44.57
2			39	40.63	37.50	36	37.50	37.50
3	16	17.39	52	56.52	48.60	45	48.90	48.91
4	42	45.65	57	61.95	52.29	49	53.26	52.69
5	46	49.46	45	48.39	43.69	41	44.08	43.62
6	51	47.66	69	64.49	54.76	61	57.01	54.46
7	38	40.00	50	52.63	42.02	48	50.53	41.74
AAM			51	53.07	46.15	46	47.71	46.21
APM	39	40.03	54	56.79	48.27	49	50.86	48.28

¹AAM, average for all milks.

²APM, average for paired milks.

³Figures for unpaired milks in this column are based on average for paired raw milks.

TABLE VIII--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recom- mended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo- rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo- rated
Acid Precipitable Phosphorus Removed by Centrifuging								
1								
2			7	7.29	6.73	7	7.29	7.29
3	10	11.96	13	14.13	12.15	11	11.96	11.96
4	28	30.43	12	13.04	11.01	11	11.96	11.83
5	15	16.13	0	00.00	00.00	0	00.00	00.00
6	24	22.43	25	26.31	19.84	20	18.68	17.86
7	9	9.47	12	12.63	10.08	10	10.33	8.69
AAM ¹			11	12.23	9.97	10	10.04	9.77
APM ²	17	18.08	12	13.22	10.61	10	10.58	10.27
Non-Precipitable Phosphorus Removed by Centrifuging								
1								
2			31	32.29	29.81	29	30.21	31.52
3	6	6.52	39	42.39	36.45	34	36.96	36.96
4	14	15.22	45	48.91	41.28	38	41.30	40.86
5	31	33.33	46	49.46	44.66	42	45.16	44.68
6	28	26.17	44	41.12	34.92	41	38.32	36.61
7	29	30.53	39	41.05	32.77	38	40.00	33.04
AAM			42	42.54	36.65	37	38.66	37.28
APM	21	22.35	43	44.59	38.02	38	40.35	38.43

¹AAM, average of all milks. ²APM, average paired milks.

³Figures for unpaired milks in this column are based upon the average for the paired raw milks.

TABLE IX
THE DISTRIBUTION OF CALCIUM IN RAW AND EVAPORATED MILKS

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recommended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo-rated
Total Calcium in Whole Milk								
1			152	113.43	100.00	137	102.24	100.00
2			131	97.76	100.00	121	90.30	100.00
3	121	100.00	127	104.96	100.00	109	90.08	100.00
4	126	100.00	126	100.00	100.00	107	84.92	100.00
5	128	100.00	137	107.03	100.00	125	97.66	100.00
6	144	100.00	152	105.55	100.00	135	93.75	100.00
7	147	100.00	153	102.68	100.00	148	93.15	100.00
AAM ¹			140	104.47	100.00	126	94.04	100.00
APM ²	134	100.00	139	104.04	100.00	125	93.15	100.00
Serum Calcium								
1			50	37.21	32.85	45	33.58	32.85
2			42	31.34	32.06	39	29.10	32.23
3			35	28.93	27.56	30	24.79	27.52
4	54	42.86	56	44.44	44.80	47	37.30	43.92
5	55	42.97	31	24.22	22.63	28	21.87	22.40
6	50	34.72	45	30.56	29.61	40	27.78	29.61
7	51	34.23	54	36.24	35.29	52	34.90	35.13
AAM			45	33.29	32.12	40	29.90	31.95
APM	53	38.69	47	33.61	33.08	42	29.33	32.77
Colloidal Calcium of Whole Milk								
1			102	76.11	67.10	92	76.03	67.15
2			89	66.42	67.94	82	67.75	67.77
3			92	76.03	72.44	79	65.29	72.48
4	72	57.14	70	55.55	56.00	60	47.62	56.07
5	73	57.03	106	82.81	77.37	97	75.78	77.60
6	94	65.28	107	74.31	70.39	95	65.97	70.37
7	98	65.77	99	66.44	64.70	96	64.43	64.86
AAM			95	71.09	71.09	86	66.13	65.19
APM	84	61.31	95	69.78	69.89	87	63.45	67.23
Calcium in Centrifugate								
1			73	54.48	48.03	65	49.25	51.17
2			75	55.97	57.25	69	51.49	57.02
3	79	65.29	40	33.06	51.50	34	28.10	31.19
4	59	46.83	58	46.03	46.48	49	38.89	45.79
5	59	46.09	46	35.94	33.58	42	32.81	33.60
6	52	36.11	55	38.19	36.18	49	34.03	36.30
7	71	47.65	60	40.27	39.21	58	38.93	39.19
AAM			58	47.42	41.74	52	39.09	41.61
APM	64	48.39	52	38.70	37.37	46	34.35	37.21

¹AAM, average for all milks. ²APM, average for paired milks.

³Figures for unpaired milks in this column are based on average for paired milks.

TABLE IX--CONTINUED

Milk Number	Raw Milk		Evaporated Milk					
			Diluted to Recom- mended Concentration			Diluted to Nitrogen Concentration of Raw		
	Mg. per 100 cc.	Per cent of Total Raw	Mg. per 100 cc.	Per cent of Total Raw ³	Per cent of Total Evapo- rated	Mg. per 100 cc. ³	Per cent of Total Raw ³	Per cent of Total Evapo- rated
Colloidal Calcium in Centrifugate								
1			23	17.16	15.13	21	15.67	15.33
2			33	24.63	25.19	30	22.39	24.79
3			5	4.13	3.94	4	3.31	3.67
4	5	3.97	2	1.59	1.60	2	1.59	1.87
5	4	3.13	15	11.72	10.95	14	10.94	11.20
6	2	1.39	10	6.94	6.59	9	6.25	6.67
7	20	13.42	6	4.03	3.92	6	4.03	4.05
AAM ¹			13	10.03	9.62	12	9.17	9.65
APM ²	8	5.48	8	6.07	5.76	8	5.70	5.92
Total Calcium Removed by Centrifuging								
1			79	58.95	51.95	71	52.99	46.71
2			56	41.79	42.75	52	38.81	39.69
3	42	34.71	87	71.70	68.50	75	61.98	59.06
4	67	53.17	68	53.97	54.40	58	46.03	46.40
5	69	53.91	91	71.09	66.42	83	64.84	60.58
6	92	63.89	97	67.36	63.81	86	59.72	56.58
7	78	52.35	93	62.42	60.78	90	60.40	58.59
AAM			82	64.93	58.38	74	54.97	52.55
APM	69	51.61	87	65.35	62.78	78	58.59	56.29

¹AAM, average of all milks. ²APM, average paired milks.

³Figures for milks in these columns that have no raw pairs are based on the average for the paired milks.

facture, had to be selected as the factor representing differences in the milk at a lesser concentration. Thus, when the nitrogen concentration of evaporated milk is equal to the nitrogen concentration of the raw milk, the concentrations of the calcium and phosphorus of the evaporated will also be equal to their concentrations in the raw milk unless the manufacturing process has caused changes in their concentrations. If changes resulted only because of the heat treatment of the milk, they would be more evident in evaporated milks with the greater water content. The assumption that the lesser concentration merely distributes the particles through a greater volume involves the assumption that the ratio of partition of the elements will not differ from that which is found at the higher concentration. It is recognized that the addition of more solvent could change the ratios slightly. On the other hand, the volume of water which it is necessary to add to give the desired concentration is small and if the ratio should not remain absolutely constant, the difference should be slight and should certainly not alter the direction of the changes observed at the lesser concentration.

Upon these assumptions, then, calculations were done to determine what the percentage of the elements would be in evaporated milks if they were diluted to the nitrogen concentration of their paired raw milks. The computations were as follows: the ratio of the nitrogen in the raw milk to the nitrogen in the evaporated milk was first determined, and the factor obtained in this way was used as the reduction factor for each element in each fraction of the evaporated milk. Thus if the raw milk had 489 milligrams nitrogen per 100 cubic centimeters and the evaporated milk had 571, the reduction factor would be $\frac{489}{571} = 0.8571$. The figure for the nitrogen of the evaporated milk was then multiplied by that factor. $571 \times 0.857 = 489$ milligrams nitrogen in evaporated milk at the new dilution. There are 479 milligrams of acid-precipitable nitrogen per 100 cubic centimeters. When this is multiplied by the factor 0.8571 it is found that 410 milligrams per 100 cubic centimeters is the figure for the acid-precipitable nitrogen at the new dilution. In like manner all the other figures are reduced by multiplying each one by its factor.

In order to facilitate the discussion the averages of the paired milks will always be referred to in the interpretation of the data. Reference to the tables will reveal that they do not

vary markedly from the averages for all milks, but since they are derived from data from analysis of real milks rather than an average which represents a theoretical milk, they should be more reliable. Both dilutions of the evaporated milks will be considered.

Nitrogen

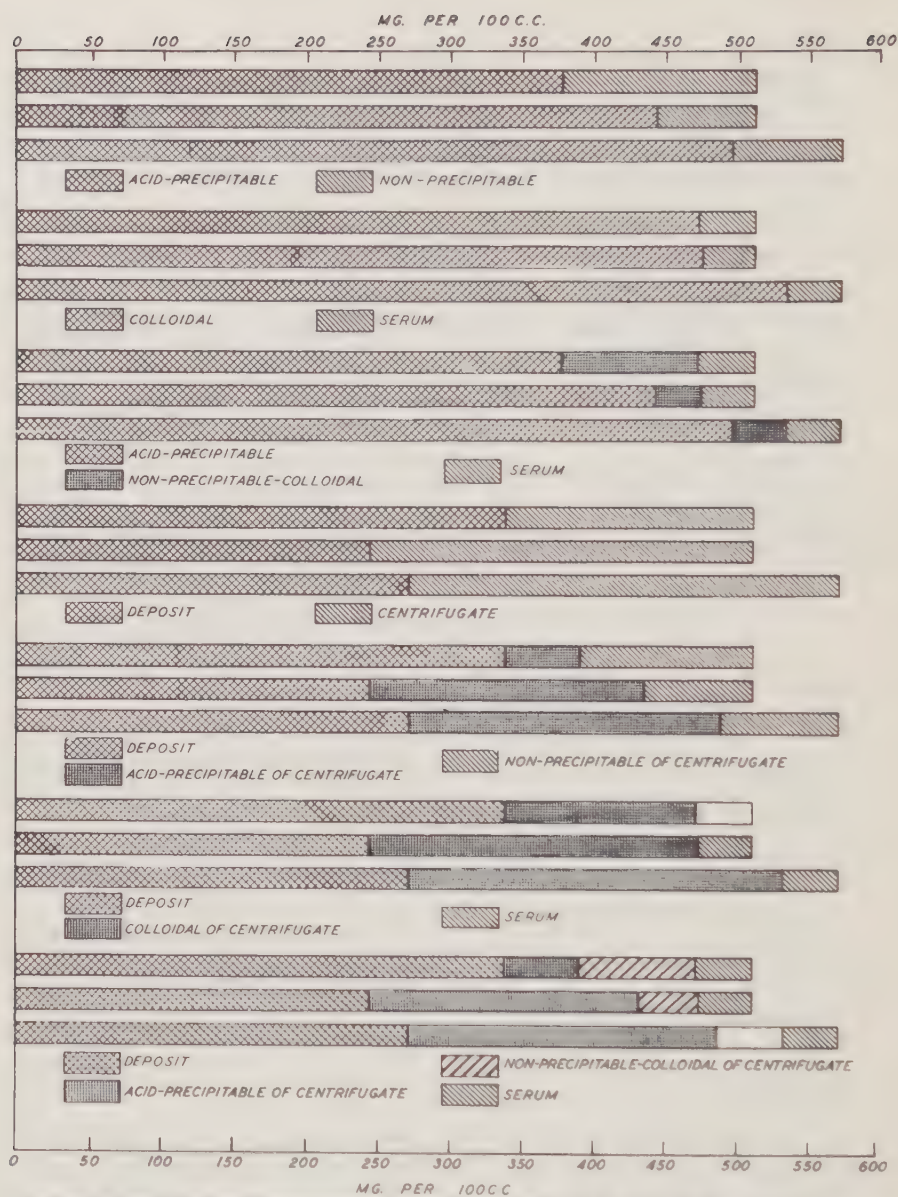
Total nitrogen.--Inspection of Table VII shows that the average total nitrogen of the evaporated milk at the recommended dilution exceeds that of the raws by 11.94 per cent. While this is small, it is possible that in certain dietary regimes this additional nitrogen could be very important. The raw milks are closely alike in their nitrogen content and fall well within the range for the nitrogen content of milk reported in the literature. The evaporated milks are also closely alike. Figures for evaporated milk at this dilution have not been found, but the figures reported for the undiluted evaporated milk show that these figures are within the range expected for total nitrogen.

By computation, as described before, the figures for the evaporated milk nitrogen at the lesser concentration are identical with the figures for the raw milk nitrogen.

The acid-precipitable and non-precipitable nitrogen.--The nitrogens of the acid-precipitable fraction and the non-precipitable fraction are considered together, since they make the whole. At the recommended dilution, the evaporated milk fractions show an increase of 27.7 per cent of acid-precipitable nitrogen, which is also reflected as a loss of 27.7 per cent of non-precipitable nitrogen at the same concentration. This shift of nitrogen from the non-precipitable to the precipitable fraction is due to the coagulation of the albumin which remains insoluble in acid. The high figure obtained at the recommended dilution is reduced to 12.9 per cent when the average for the milks at the lesser concentration is used.

In the raw samples the acid precipitable nitrogen is 73.6 per cent of the total, while in the more concentrated evaporated milk the percentage is 96.8 of the total, being reduced to 86.6 per cent in the evaporated at the lesser concentration. These relationships are graphically represented in Figure 1.

Serum nitrogen.--At the recommended dilution the evaporated milk shows a gain of 6.86 per cent of truly soluble nitrogen, but at the lesser concentration there is a loss of 2.5 per



THE FIRST COLUMN IN EACH GROUP REPRESENTS MG. OF NITROGEN PER 100 C.C. OF RAW MILK, THE SECOND, EVAPORATED MILK DILUTED TO THE NITROGEN CONCENTRATION OF THE RAW MILK AND THE THIRD, THE EVAPORATED MILK DILUTED TO THE RECOMMENDED DILUTION.

FIG. 1.—THE RELATIONSHIP OF THE AVERAGE NITROGEN FRACTIONS TO EACH OTHER AND TO THE AVERAGE WHOLE MILK

cent, a loss which is in line with the reports of other workers that there is a loss of soluble nitrogen when milk is autoclaved. Losses are also reported at temperatures considerably below the boiling point, so it is surprising that the loss of truly soluble nitrogen should be so small. Perhaps the small loss can be attributed to the nature of the filter. Although it is usually considered that albumin will pass through a filter of the type used, it is also known that the quantity passing through is dependent upon the size of the pores of the filter. The porosity of the filters was not determined, so it is probable that the pores of the particular ones used were small enough to retain the so-called soluble nitrogen. Moreover, as Van Slyke pointed out, perhaps much of the soluble nitrogen is absorbed by the colloidal protein.

Soluble nitrogen represents 7.4 per cent of the total nitrogen of the raw milk and 6.9 per cent of the total nitrogen of the evaporated milk at the recommended dilution which becomes 7.0 per cent at the greater dilution. This is graphically represented in Figure 1. Although there is an apparent loss when the average percentages are compared, if the averages of the weights are compared it is seen there is only one milligram difference between the raw and evaporated milks. This is a rounded figure and therefore brings the average of the two milks closer together. By any method of computation the difference is so small that it can have no significance.

Colloidal nitrogen.--All the nitrogen that is removed from milk by filtration is colloidal, so that the Pasteur-Chamberland residue represents all of the colloidal material of the milk. This residue plus the serum nitrogen makes the whole milk, as seen in Figure 1. The total colloidal nitrogen represents 92.65 per cent of all the nitrogen of the raw milk, 93.4 per cent of all the nitrogen of the evaporated milk at the recommended dilution and 90.4 per cent of all the nitrogen of the less concentrated evaporated milk.

Now all the acid-precipitable nitrogen is colloidal, so if it is subtracted from the total colloidal nitrogen another colloidal fraction is found, the non-precipitable-colloidal nitrogen. Another method of calculating the non-precipitable-colloidal fraction is to subtract the serum nitrogen from the non-precipitable fraction, which is also termed the acid filtrate. At both

concentrations the evaporated milks have less of this particular fraction of nitrogen than the raw milks. This is expected, for this fraction of the raw milks contains the so-called soluble proteins which are coagulated when they are heated. This non-precipitable-colloidal fraction represents 18 per cent of the total and 20 per cent of the colloidal nitrogen of the raw milks; in the more concentrated evaporated milks it represents 5.8 per cent of the total and 6.3 per cent of the colloidal nitrogen, while for the more dilute evaporated milks these figures are 5.6 and 5.8 per cent, respectively.

Centrifugate nitrogen.--When the milks are centrifuged large quantities of the colloidal materials are removed from the fluid portion as a solid deposit. The evaporated milks lose less by this treatment than do the raw milks. The nitrogen retained in the fluid centrifugate of the raw milk is only 33.6 per cent of the total original nitrogen, while the evaporated milk at either concentration retains 51.8 per cent of its original nitrogen. The centrifugate fraction becomes important because through its analysis it is seen that the size of the colloidal particles has been reduced during the process of manufacture. If there were no change in particle-size of the evaporated product, the same weight of nitrogen would be deposited from it that is deposited from the raw. Now since albumin coagulates during the heating process, it would seem reasonable to expect more deposit from the evaporated milk. However, since that is not what has been observed, it is probable that the process of homogenization subdivides the albumin and other colloidal particles so finely that they resist deposition by centrifugal force.

The acid-precipitable and non-precipitable nitrogen of the centrifugate.--When the centrifugate is treated with acid, the nitrogenous constituents are separated into two fractions, the precipitable and the non-precipitable fractions. It has been shown that 73.6 per cent of the nitrogen of raw milk and 86.6 per cent of the nitrogen of evaporated milk was precipitated in this way. But in the raw centrifugate only 29.5 per cent of the nitrogen is precipitable while in the evaporated centrifugate 72.1 per cent is precipitable. This means, in other words, that 86.5 per cent of the total acid-precipitable nitrogen of the raw milk is removed by centrifuging, while only 43.2 per cent of the acid-precipitable nitrogen of evaporated milk is deposited by like

treatment. This adds more evidence to that already mentioned of the colloidal-size reduction in the process of manufacturing evaporated milk.

When the non-precipitable nitrogen of the evaporated centrifugates is compared with the total non-precipitable nitrogen it is seen that the average in the filtrate of this fraction is higher than the average of the whole. This relationship is irregular for the individual milks but three of the five paired evaporated milks do show this increase. The raw centrifugates either do not change or lose non-precipitable nitrogen. It seems paradoxical for the fraction to contain more of one particular kind of nitrogen than the whole. A probable explanation is that the process of manufacturing reduces the power of adsorption of the acid-coagulum. This does not show up when the whole milks are compared because of the loss of the coagulated albumin from the evaporated fraction, but when the larger portion of the acid-precipitable nitrogen is removed by centrifuging, that nitrogen which was formerly held by the precipitable coagulum is left in the acid-filtrate.

Colloidal nitrogen of the filtrate.--As with the whole milks, colloidal nitrogen of the centrifugates may be found by subtracting the serum nitrogen, which is a part of the centrifugate nitrogen, from the whole nitrogen. The tables show that 26.8 per cent of the total colloidal nitrogen of the raw and 49.1 per cent of the total nitrogen of the evaporated milks are retained in the centrifugates. It would follow, naturally, that if less total nitrogen were retained in the raw centrifugates than in the evaporated centrifugates, the same relationship would exist for the colloidal nitrogen of the centrifugates, for only colloidal nitrogen is removed by centrifuging. This becomes another point in the evidence that the process of manufacture does affect particle-size.

The non-precipitable-colloidal nitrogen fraction of the evaporated centrifugate, shows the same paradoxical increase of nitrogen that is observed in the non-precipitable fraction of the whole centrifugate. This results from the fact that, if the explanation offered for the phenomena in the non-precipitable fraction is correct, the extra nitrogen in the non-precipitable fraction is wholly colloidal for the total soluble nitrogen will not vary.

The deposit nitrogen.--Since the deposit, or residue, nitrogen of the milks is obtained by difference, it will be readily observed from the data that when losses from any fluid fraction are found, those losses will be reflected as gains in the complementary solid fraction. Remembering that the deposit is colloidal material, it may be separated into acid-precipitable and non-precipitable fractions, which are readily calculated by subtracting the precipitable and non-precipitable nitrogen of the centrifugates from their whole fractions to find the value of these fractions of solid material. Detailed analysis of the deposit data only confirms the conclusions already pointed out.

Summary.--Figure 1 graphically summarizes the relationships of the average nitrogen fractions to each other and to the whole milk. It is profitable to fit the fractions together to make up the whole milk. Since there are so many fractions which overlap each other in so many ways, a number of different combinations may be made to build the whole milk. The chart shows how each combination may be made for the average raw and the two dilutions of evaporated milk. The whole milk may be built up by combining (a) the acid-precipitable and non-precipitable fractions, (b) the colloidal and serum fractions, (c) the acid-precipitable, non-precipitable-colloidal and serum fractions, (d) the deposit and the centrifugate, (e) the deposit and the acid-precipitable and non-precipitable fractions of the centrifugate, (f) the deposit and the colloidal and soluble (serum) fractions of the centrifugate, (g) the deposit and the acid-precipitable, non-precipitable-colloidal and soluble fractions of the centrifugate.

From these data the following conclusions may be drawn:

- 1) At the concentrations at which evaporated milk is commonly used there is 11.9 per cent more nitrogen in it than in raw milk. The degree of dilution can cause this quantity to vary.

- 2) Changes in the physical condition of milk are produced in the process of manufacturing evaporated milk, for 15 per cent of the nitrogen is coagulated during the process.

- 3) Further evidence of a physical change is offered by the fact that the supercentrifuge cannot deposit as much colloidal nitrogen from evaporated milk as it can from raw milk. If coagulation involves agglutination processes then it might be expected that the colloidal particles of the evaporated milk would be

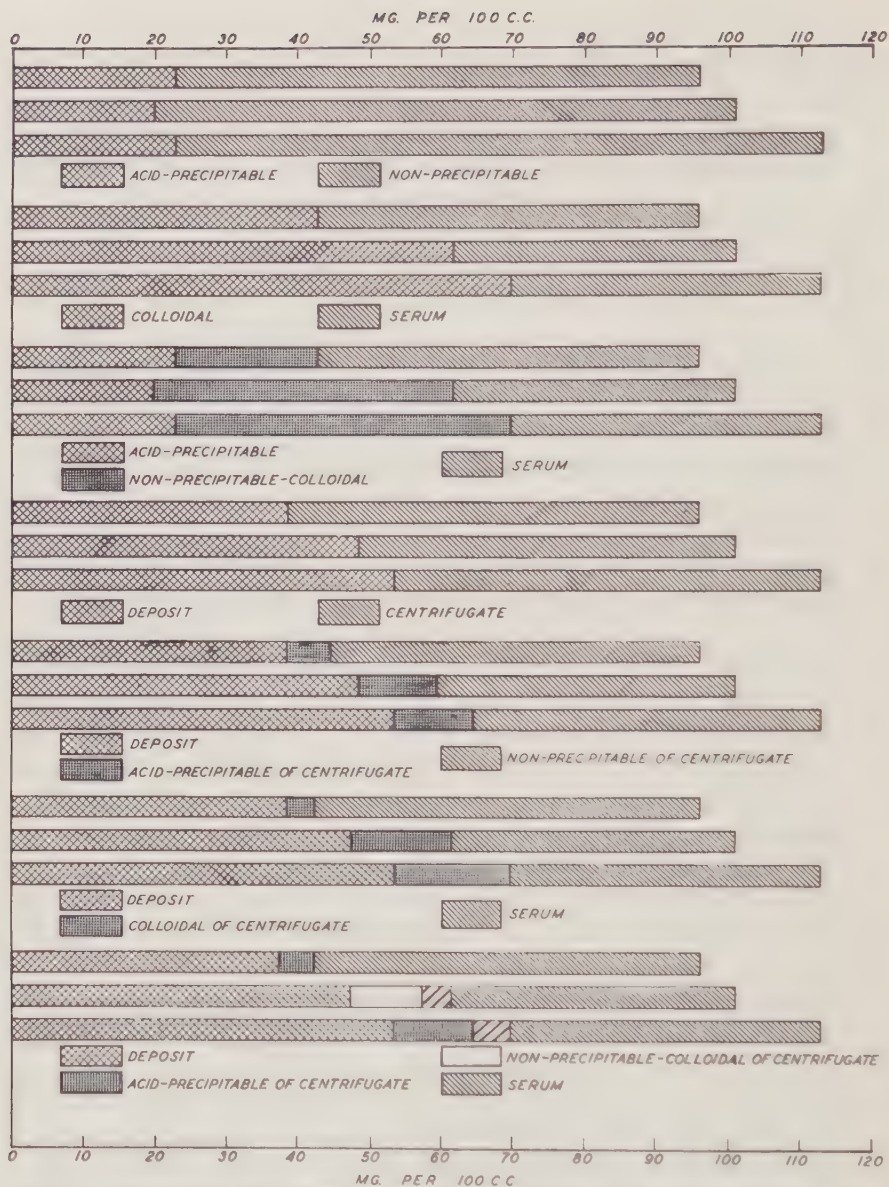
larger than those in the raw milk. If this were the final condition then more colloidal material could be deposited from the evaporated milk. Since it cannot be deposited, it is probable that the homogenization of the milk not only subdivides the fat globules, but the colloidal nitrogenous aggregates as well.

4) Only an insignificant amount of soluble nitrogen is rendered insoluble during the manufacturing process.

Phosphorus

Total phosphorus.--Table VIII contains the data which show the distribution and relationships of phosphorus in raw and evaporated milks. At the recommended dilution there is 17.7 per cent more phosphorus in evaporated than in raw milk. When the percentage is computed for the more dilute milk there still is an average of 5.5 per cent more phosphorus than is in the raw milk. Comparison of the individual milks shows that the increases are probably within experimental error except for the seventh milk which still is 21 per cent higher in phosphorus than the raw. If that sample is omitted from the calculations, the average difference then becomes 1.7 per cent. At one time condensaries added stabilizers to milks, and since many of them were phosphate, it seemed reasonable to look to that practice for the increase in phosphorus encountered in sample seven. Moreover, it was necessary to use stabilizers more generously in spring milk than in fall or winter milk and this milk was collected in April. However, since this practice has been discontinued, the source of the extra phosphorus has not been found. It is surprising of course to find an increase in phosphorus in the more dilute milks and it is almost as surprising to find no loss during the manufacture of evaporated milk since it has been so generally believed that heat causes a precipitation of phosphorus. It was seen in the case of colloidal nitrogen that the processing of the milk resulted in a more finely dispersed nitrogen phase. Now the more finely divided the colloidal material, the less is the effect of gravity in producing settling. It therefore seems that whatever the shifts in the condition of phosphorus, it remains colloidally stable and is not precipitated from the milk.

Acid-precipitable and non-precipitable phosphorus.--The sum of these two fractions equals the whole (Figure 2) and should be considered together. Whereas most of the nitrogen was pre-



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FIG. 2.—THE RELATIONSHIP OF THE AVERAGE PHOSPHORUS FRACTIONS TO EACH OTHER AND TO THE AVERAGE WHOLE MILK

precipitated, by far the greater portion of the phosphorus is soluble in acid, only 24.4 per cent of the raw and 20 per cent of the evaporated being precipitable. Van Slyke and Bosworth have reported (39) that 0.71 per cent of casein is phosphorus. When the average total phosphorus is calculated on that basis, it is found that 17.7 per cent of the total is in chemical union with the casein, leaving a minute percentage which can be considered as being absorbed or otherwise actually insoluble in acid.

Serum phosphorus.--The soluble phosphorus of the raw milks averages 55.2 per cent of the total. This is in close agreement with Van Slyke and Bosworth's figure of 51.4 which has been calculated by the writer from the averages of several milks reported by them. Only 37.6 per cent of the phosphorus of the evaporated milks is soluble however. This means that 26.06 per cent of the serum phosphorus has been removed from the serum. In the work reported in Table III, only Diffloth secured a figure that is comparable to this.

Colloidal phosphorus.--There is more colloidal phosphorus in evaporated milk than in raw milk, for 63.05 per cent of evaporated milk is found in the colloidal fraction and only 45.4 per cent of the total raw. The quantity of colloidal phosphorus contrasts interestingly with colloidal nitrogen, which composed approximately 90 per cent of the total. By accepting Van Slyke and Bosworth's figure of 0.71 per cent of phosphorus in casein and calculating the casein phosphorus as per cent of total colloidal, it can be seen that only 38 per cent of the colloidal phosphorus of the raw milk is in the casein, and only 27 per cent of the evaporated milk phosphorus is in the casein. This leaves the greater part of the colloidal phosphorus to be considered as inorganic colloidal phosphorus.

Non-precipitable-colloidal phosphorus.--Like colloidal nitrogen, colloidal phosphorus may also be separated into two fractions, (a) that which is precipitable with acid and (b) that which is not precipitable. This latter fraction is inorganic colloidal phosphorus, but may not be the total inorganic fraction, for there is that very small portion that is precipitable but not in chemical union with casein, which may also be inorganic. But this non-precipitable fraction constitutes not less than two-thirds of the colloidal phosphorus fraction of the evaporated milk or one-half of that of raw milk. The process of manu-

facture, therefore, does increase the total colloidal inorganic phosphorus, either by precipitating the soluble phosphorus from the serum, which seems probable, or by hydrolyzing the casein.

Centrifugate phosphorus.--Approximately half of the total phosphorus of both types of milk is removed by centrifugation, although a measurable difference is found between the centrifugates of the two milks, the evaporated centrifugate containing 8 per cent less phosphorus than the raw. It will be recalled that there was 1.7 times as much nitrogen in the evaporated centrifugate as in the raw. This only points again to the fact that colloidal nitrogen and phosphorus are not in chemical union.

Acid-precipitable phosphorus of the centrifugate.--As has been mentioned before, the centrifugate was separated into two subfractions by the same techniques that were employed in fractioning the whole milk. This gives a more detailed picture of the effect of manufacture upon the distribution of the phosphorus in that portion of milk that contains only part of the colloidal but all of the soluble phosphorus. The acid-precipitable fraction of the centrifugate is 9.8 and 19 per cent respectively of the raw and evaporated centrifugate phosphorus, or 26 and 55 per cent respectively of the acid-precipitable phosphorus of the whole raw and evaporated milks. The quantity of phosphorus which is combined cannot be determined for the evaporated centrifugate, because there is no method of separating the casein from other precipitable protein in the precipitable fraction of the centrifugate. The fact that different percentages of acid-precipitable nitrogen and phosphorus are removed from the same kind of milk only bears evidence to what is already known; that they are not chemically combined and their compounds have different molecular weights. That different percentages of phosphorus are lost from the different kinds of milks adds evidence to that already observed that the colloidal character of the evaporated milk is altered.

Non-precipitable phosphorus of the centrifugate.--The evidence that is based upon the data for the precipitable fraction is only supplemented by evidence from the non-precipitable fraction. This fraction furnishes 90.2 per cent of the phosphorus of the raw centrifugate and 81 per cent of that of the evaporated centrifugate, which constitutes 71 and 54.5 per cent of the respective totals of non-precipitable phosphorus.

Colloidal phosphorus of the centrifugate.--It is interesting to find that practically none of the phosphorus of the raw centrifugate is in colloidal dispersion. Either Table IX or X shows that the figure for total soluble phosphorus, all of which is present in the centrifugate, exceeds the figure for total phosphorus in the raw centrifugate in two cases. However, the differences are small and are probably within experimental error. In those centrifugates that have some colloidal phosphorus the quantity is small, equaling in fact the negative differences in the other two cases and furnish no decisive evidence that there is colloidal phosphorus in the raw centrifugate. However, the figures for colloidal phosphorus of the evaporated centrifugates, although low, are sufficiently high to warrant the statement that there is some colloidal phosphorus retained in the evaporated centrifugate. This is another evidence of colloidal change during the process of manufacture.

Non-precipitable-colloidal phosphorus of the centrifugate.
--Of course if there is no colloidal phosphorus in the raw centrifugate there can be no non-precipitable-colloidal phosphorus in it. The fact, however, that subtraction of the total soluble phosphorus from the non-precipitable phosphorus always shows that there is slightly more truly soluble phosphorus than non-precipitable phosphorus in the centrifugate, presents a paradoxical situation, for the truly soluble represents a sub-fraction of the non-precipitable fractions. The situation can be explained only by assuming that the truly soluble fraction has some phosphorus added to it that has not been added to the centrifugate. It is probable that such a condition occurred, for due to the difficulty of cleaning the candle of the Pasteur-Chamberland filter, it is possible that a small quantity of the ash from the previous filtration may have been left in the candle. If this were dissolved in the serum as it passed through the filter, the explanation is found. While, if this is the true situation, it introduces a slight error in the percentages of the element in the serums, it is believed that the relative results are not affected in any way, for every serum would be equally affected, thus not changing the relative relationships. The situation shows up only when the two fractions from the same milk, which contain only truly soluble nitrogen, but separated from the whole by different techniques are compared. It is not observed when the two similar fractions

of the evaporated milks are compared for the evaporated centrifugates contain enough colloidal phosphorus to mask the effect of the very small increases in soluble phosphorus. Comparison of the non-precipitable-colloidal fractions of the two types of milk adds still further evidence that the colloidal condition of phosphorus is stabilized during the manufacturing process.

Deposit phosphorus.--The phosphorus removed by centrifuging complements the phosphorus in the centrifugate. This may be seen in Figure 2. The raw milks deposited 41 per cent and the evaporated milks 45 per cent of their phosphorus content in the residue from the centrifuge. This, like the colloidal phosphorus remaining in the centrifugate can be separated into acid-precipitable and non-precipitable fractions. Thus, 45 per cent of the raw fraction is acid-precipitable while 55 per cent is non-precipitable. The evaporated fraction is made up of 22.5 per cent of acid-precipitable and 77.5 per cent of non-precipitable phosphorus. This is coincidental evidence with that obtained from the centrifugate fractions that there is no difference in the stability of the colloidal phosphorus constituent during the manufacturing process.

Summary.--Figure 2 summarizes graphically the material presented. The fractions are arranged in columnar order in such a way that when added together they will represent the whole milk phosphorus.

From the data presented for phosphorus the following conclusions seem warranted:

- 1) At the concentrations recommended for family use there is 17 per cent more phosphorus than is found in an equal volume of raw milk. When diluted to the same water concentration of the raw milk there is no loss of phosphorus.

- 2) There is a fixation of the serum phosphorus during the manufacturing process, but since there is no total loss this results in increasing the colloidal phosphorus of the whole.

- 3) Colloidal phosphorus constituents are no more finely subdivided in evaporated than in raw milk.

Calcium

Fewer calcium fractions and sub-fractions can be separated from whole milk than nitrogen or phosphorus fractions because there is complete hydrolysis of calcium-caseinate when it is

treated with acid and all the calcium is found in the acid-filtrate. Therefore eight fractions which are dependent upon this reaction are not obtainable for calcium; acid-precipitable and non-precipitable and non-precipitable colloidal fractions of the whole milk and of the centrifugate and the acid-precipitable and non-precipitable fractions of the deposit.

Total calcium.--Although some calcium is precipitated during the manufacture of evaporated milk, at the dilution that is recommended there is still 4 per cent more in it than in the raw milk. When the percentage is calculated on the basis of equal nitrogen concentration in each milk, there appears to have been a 6.8 per cent loss of total calcium. This figure is not far different from that obtained by Brehaut, Purvis and McHattie who autoclaved milk for half an hour and found a loss of 5.3 per cent.

Serum calcium.--Few studies (Table III) have shown decisively that serum calcium is lost when milk is heated, unless the temperature of heating reaches 100°C. or above. The evaporated milks, at both dilutions in this study, had an average of 21 per cent less calcium in the serum than did the raw serum. If the soluble loss were responsible for the total loss, it would amount to an 8.1 per cent loss. Now the total loss was only 6.85 per cent, so that although calcium was lost from the fraction it was not completely lost from the product. Part of the original serum calcium, then, is held by adsorption or in suspension in the colloidal fraction of evaporated milk.

Colloidal calcium.--At both dilutions, there appears to be an increase in the colloidal calcium of evaporated milk, which is the result to be expected when the loss of soluble calcium is greater than the total loss. An increase in colloidal phosphorus and nitrogen has been interpreted as a stabilization of the colloidal character of the constituents. This interpretation is still supported by the increase of colloidal calcium, even though that increase is small.

Centrifugate calcium.--There is 28 per cent less calcium in the evaporated centrifugate at comparable dilutions than in the raw centrifugate. Now, nitrogen and phosphorus were higher in the evaporated centrifugate than they were in the raw centrifugate. Since the centrifugate contains all the serum calcium as well as some colloidal calcium and since the serum calcium loss can account for more than the total loss, it would be expected

that the loss would also appear in the centrifugate. But the centrifugate loss is greater than the serum loss. In explanation, reference is made again to the difficulty of cleaning the filter, and the possibility of some soluble calcium being picked up from the filter as the serum passes through it. That would result in the serum having apparently lost less calcium than the centrifugate although the total calcium picked up does not bring the serum calcium up to the total centrifugate calcium. Again it must be pointed out that this only affects the magnitude of the results and not the direction.

Colloidal calcium in centrifugate.--Despite the fact that the loss of total calcium from the evaporated centrifugate is greater than that from the raw, the colloidal calcium remains constant, and very low, representing 5 to 6 per cent of the total calcium of either type of milk. This fairly well parallels the phosphorus condition, which showed that practically all the centrifugate phosphorus is soluble.

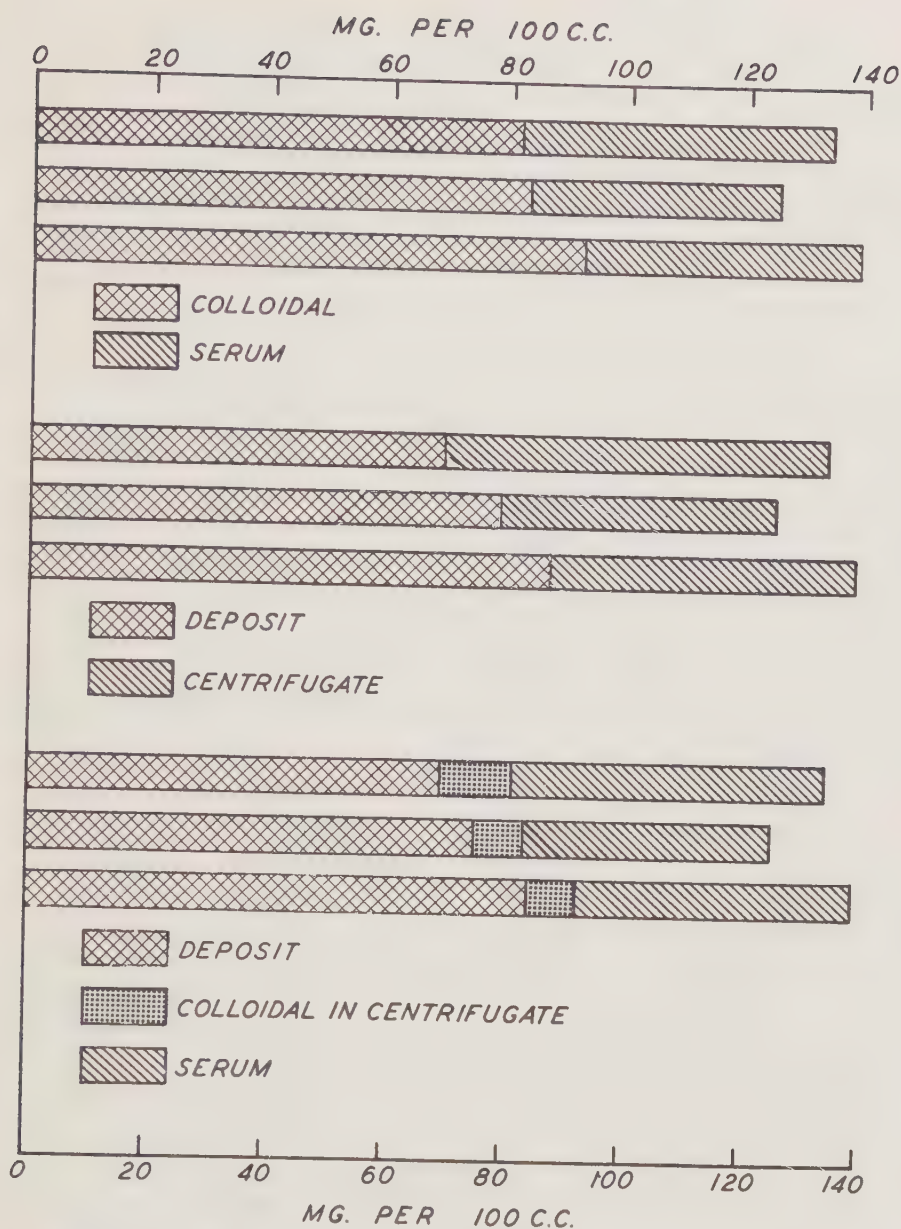
Deposit calcium.--Like deposit phosphorus and nitrogen, there is less deposit calcium in the evaporated milk than in the raw. This condition has been interpreted in each case to mean that the colloidal particles are stabilized, probably by being more finely subdivided in the evaporated product.

Summary.--Figure 3 represents the average distribution of calcium in the raw and evaporated milks. The data in Table IX and in Figure 3 justify the following conclusions:

- 1) There is slightly more calcium in evaporated milk at the dilutions recommended for its use than there is in raw milk. However, when equal volumes of water are contained in the paired milks, it becomes apparent that the manufacturing process causes losses in calcium.

- 2) The total calcium loss may be from the soluble fraction of the milk, but does not account for the total shift in the serum calcium equilibrium, for more soluble calcium is lost than total. Some of the soluble element must have been adsorbed or held in colloidal suspension.

- 3) Colloidal calcium constituents are no more finely divided in raw milk than in evaporated milk, for almost equal percentages of colloidal calcium are lost by centrifugation.



THE FIRST COLUMN IN EACH GROUP REPRESENTS MG. OF CALCIUM PER 100 C.C. OF RAW MILK; THE SECOND, EVAPORATED MILK DILUTED TO THE NITROGEN CONCENTRATION OF THE RAW MILK, AND THE THIRD, THE EVAPORATED MILK DILUTED TO THE RECOMMENDED DILUTION.

FIG. 3.—THE RELATIONSHIP OF THE AVERAGE CALCIUM FRACTIONS TO EACH OTHER AND TO THE AVERAGE WHOLE MILK

Hydrogen Ion Concentration and Buffer Values

Hydrogen ion concentration expressed as pH values and titration data have been recorded for scattered milks and centrifugates in Table X, which shows that evaporated milks have lower

TABLE X
TITRATION AND pH VALUES OF WHOLE MILK AND CENTRIFUGATES

	pH		No. cc. 0.1N Base to Change pH of 100 cc. to pH 8.0 or no. cc. 0.1 Acid to Change pH to 3.4	
	Raw	Evapo-rated	Raw	Evaporated
1 Whole		6.3		
2 Whole		5.9		23.4 (base)
Centrifugate		4.8		41.36 (base)
3 Whole	6.7	6.3	12.3 (base)	14.0 (base)
Centrifugate	6.1	6.4	20.0 (base)	13.5 (base)
4 Whole	6.7			
5 Whole		6.0		105.36 (acid)
Centrifugate		6.0		51.52 (acid)
6 Whole	6.5	6.1	91.26 (acid)	106.64 (acid)
Centrifugate		5.5		52.99 (acid)
7 Whole	6.4	6.0	91.79 (acid)	103.53 (acid)
Centrifugate		5.6		50.90 (acid)

pH values than raw milks. This is in line with the work of Orla-Jenson and Plattner (25) and of Whittier and Benton (46) who, though they do not agree as to the source of the acid, have shown an increase in hydrogen ion concentration when milk is heated which is dependent upon the time and temperature of heating. In every milk but one (milk 3) of those from which data were obtained the centrifugate is more acid than the whole milk. Not only is there a lower pH of the centrifugate but the titratable acidity is also increased in the centrifugate. The first milks were titrated with base to pH 8 and the evaporated centrifugate of milk 2 required almost twice as much base as its whole milk. Those

milks that were titrated with acid to pH 3.4 also showed more acid in the centrifugate for in general it required only half as much acid to bring the pH to 3.4 as it did to bring the whole milk to that pH. The titration curves in Figure 4 also show the increase in acidity of the evaporated milks. The titrations from which the curves are plotted were done with base, bringing the pH to pH 8, or more. These curves are practically superimposable, so nearly identical are the slopes of each. The chief difference in the curves is their point of origin.

General Relationships of Calcium, Phosphorus, and Nitrogen

Feeding experiments with human beings, as well as with experimental animals, have frequently shown that evaporated milk is more efficiently utilized than is raw, pasteurized or boiled milk. It has also been shown to be more completely digested in vitro by trypsin (Wallen-Lawrence and Koch, 41). Now it has often been generally accepted that the utilization of minerals is dependent upon their solubility. However, the calcium and phosphorus in raw milk are more soluble than in evaporated milk, yet they are better utilized in the latter. The ease of digestion of protein has frequently been attributed to the softness of the curd formed in the stomach. Wallen-Lawrence and Koch (41) believe the increase in trypsin digestibility to be a "function of the temperature to which the milk is heated and the length of time it is heated."

The data of this study show that the calcium, phosphorus and nitrogen all become more colloiddally stable during the process of manufacturing evaporated milk. The more finely divided a colloidal material is, the more surface it will have in contact with the digestive enzymes and the greater the opportunity for complete digestion. This could, in part, account for the greater utilization of the protein. As for the better utilization of the calcium and phosphorus, there is no evidence that they must be ingested in a soluble form to be completely utilized and it is possible that improvement in the utilization of one constituent is accompanied by the better utilization of its companionate constituents. Moreover, it must not be overlooked that there is actually more calcium, phosphorus and nitrogen per unit volume of milk in evaporated milk at the usual dilution than in raw which could in-

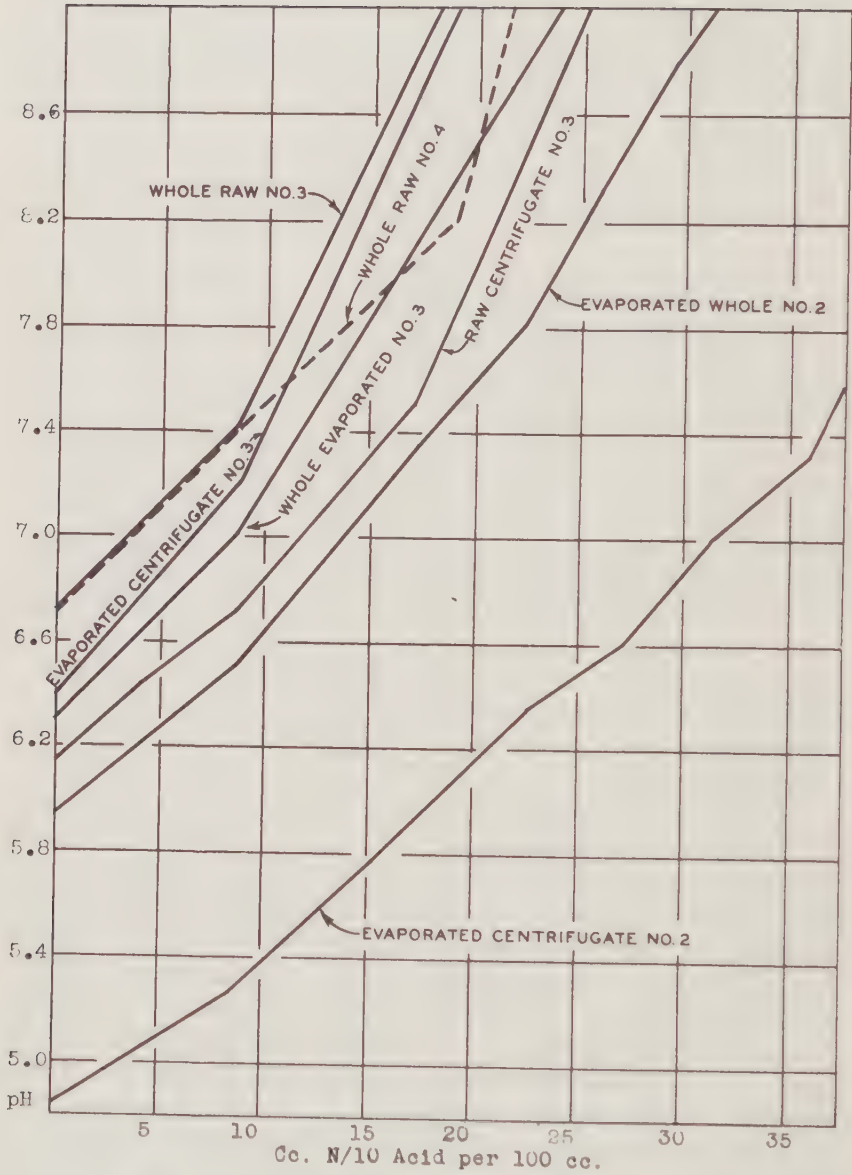


Fig. 4.--Titration Curves for Raw and Evaporated Whole Milks and for Raw and Evaporated Centrifugates.

crease the total utilization of those constituents.

SUMMARY

Some of the changes in the partition of calcium, phosphorus and nitrogen of milk that occur during the process of manufacture of evaporated milk have been studied by separating raw and evaporated milk into several fractions by using one chemical and two mechanical methods of separation and analyzing the fractions for calcium, phosphorus and nitrogen. The chemical method consisted of separating the acid soluble and insoluble constituents by precipitation with acetic acid. The two mechanical methods consisted of filtration through a Pasteur-Chamberland filter and by centrifugation in a Sharples super-centrifuge to remove the colloidal from the soluble constituents. On the basis of the data obtained, the following summary may be made:

1) The total calcium phosphorus and nitrogen is higher in evaporated milk at the concentrations recommended for its use than it is in raw milk.

2) Processing causes coagulation of considerable of the protein but does not materially affect the soluble nitrogen.

3) Centrifugation deposits less nitrogen, approximately equal quantities of phosphorus and more calcium from evaporated milk than from raw milk. The deposit from the raw milk represents 74 per cent of the colloidal nitrogen, approximately all of the colloidal phosphorus and 90.5 per cent of the colloidal calcium while the deposit from the evaporated milk represents 59 per cent of its colloidal nitrogen, 77 per cent of its colloidal phosphorus and 91 per cent of its colloidal calcium.

4) The retention of more colloidal nitrogen in the evaporated milk is indicative of stabilization of the colloidal state of the nitrogenous constituents.

5) An increase in the power to retain some of the constituents studied and a decrease in the ability to retain others during centrifugation indicates that these constituents are not in chemical union.

6) There is less phosphorus and calcium in the serum of evaporated milk than in the serum of the raw milk. The losses, however, are not proportional to each other nor to the total losses. Since there was no total loss of phosphorus, but a loss

of serum phosphorus, the net result is an increase in colloidal phosphorus. The same condition is also true for calcium, but to a lesser extent.

7) Changes in the colloidal condition of evaporated milk and an increase in the total milks solids may be partly responsible for the better utilization of evaporated milk by human beings and by experimental animals than raw milk.

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